

Effects of Dietary Lead and Cholesterol Supplementation on Hemolysis in the Sprague-Dawley Rat¹

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ABSTRACT. Hemolytic anemia has been observed in a number of organisms exposed to lead. Previous investigators have proposed that heavy metals inhibit cholesterol synthesis, which leaves insufficient cholesterol for the maintenance of cell membranes. This causes hemolysis and the release of hemoglobin and membrane associated proteins into the serum. Lead-exposed fish have displayed depressed serum cholesterol and elevated serum protein concentrations. The goals of the present investigation were to determine whether these hematological changes occur in mammals exposed to dietary lead and to test the hypothesis that lead induces a cholesterol deficiency responsible for this hemolytic effect. Adult male Sprague-Dawley rats were divided into four groups: an untreated control (group I), a group fed a 4% cholesterol supplemented diet (group II), a group exposed to lead nitrate via the drinking water (250 mg lead/liter) (group III), and a group given both lead nitrate and cholesterol (group IV). Blood samples were collected weekly for five weeks. Mean hematocrit, mean serum hemoglobin, and mean serum cholesterol concentrations were not significantly different ($p > 0.05$) in lead exposed animals. These results indicate that dietary lead exposure does not induce a cholesterol deficiency and subsequent hemolysis in the Sprague-Dawley rat.

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INTRODUCTION

Lead is one of the most toxic heavy metals found in the environment (Needleman and others 1990) and has become the most widely distributed toxic heavy metal in the urban environment as a result of its extensive use as an antiknock additive in gasoline (Brown and Hem 1984). Humans are exposed to lead by a number of routes. Exposure may occur through inhalation of ambient lead in the air (Harrison and Laxen 1981). Children increase their exposure to lead by putting toys, fingers, and other non-food items (such as paint) in their mouth (Gottlieb and Koehler 1994). Surif and Chai (1995) found that some occupational groups, such as printing press workers and bus drivers, have increased lead burdens. Humans are also exposed to lead from drinking water that passes through lead plumbing (Alexander and others 1993).

A great deal of research has been devoted to studying the effects of lead and other heavy metals upon fish, and there is a large body of literature describing the effects of lead upon the hematological parameters of fish. Tewari and others (1987) found that exposure of the freshwater fish *Barbus conchoni* to 47.4 $\mu\text{g Pb}^{2+}/\text{l}$ resulted in a decreased red blood cell count and severe anemic conditions. Dawson (1935) observed a decreased erythrocyte and eosinophil count in catfish (*Ameriurus nebulosus*) exposed to water containing 1% lead acetate, and he described an anemic condition that became more severe with increased lead exposure. Ruparella and others (1989) observed decreased serum glucose and cholesterol levels as well as increased serum protein

levels in the fish *Oreochromis mossambicus* exposed to 18 mg Pb^{2+}/l . The effects reported in these studies are consistent with those found in mammals that have been exposed to lead. For example, Aly and others (1993) reported hemolysis and anemia in a man 50 days following exposure to lead from a shotgun injury. Jehan and Motlag (1995) reported depressed cholesterol levels in erythrocyte membranes of rats intraperitoneally injected with 20 mg Pb^{2+}/kg body weight for 14 consecutive days. Zimmerman and others (1993) reported decreased resistance to oxidation in erythrocyte membranes and increased fragility of erythrocytes in Wistar rats exposed to drinking water containing 0.4% lead acetate. These studies suggest that lead has a degenerative effect upon erythrocyte membranes.

Although a relationship between serum cholesterol concentration, serum protein levels, and anemia has been observed in fish living in lead-contaminated water and in rats that have been injected with lead, it is uncertain whether these changes occur in mammals that are exposed to dietary lead. Furthermore, the mechanism by which hemolysis occurs in lead-exposed fish is not well understood. Dutta and Haghighi (1986) suggested that heavy metals inhibit cholesterol synthesis in fish. As a result, there is insufficient cholesterol for the maintenance of cell membranes. This causes hemoglobin and membrane-associated proteins to be released into the serum. The goals of the present investigation were to determine if dietary lead exposure results in hemolysis in mammals and if hemolysis is a result of a lead-induced cholesterol deficiency. A dietary lead dose of 250 mg/l was administered through the drinking water. Because adult Sprague-Dawley rats drink approximately 40 ml of water per day, this dietary lead dose approximates the daily lead exposure used by Jehan and Motlag (1995) to demonstrate that lead injections depress cholesterol

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concentrations in erythrocyte membranes. Furthermore, this dose has been used by other investigators to study physiological effects of high dietary lead exposure (Bogden and others 1991; Bogden and others 1995; Han and others 1996; Zheng and others 1996). If hemolytic anemia results from lead-induced cholesterol deficiency, then it was anticipated that serum hemoglobin and serum protein concentrations would be significantly elevated and packed cell volume significantly depressed in the lead-exposed animals. These effects could presumably be minimized by the supplementation of cholesterol through the diet.

MATERIALS AND METHODS

Thirty-two adult male Sprague Dawley rats were divided into four groups of eight animals. All groups were fed a cholesterol-free cornmeal and soybean based diet (Harlan Teklad diet 7012) and provided distilled water ad lib. Group I was the untreated control. The diet of group II was supplemented with 4% cholesterol. Cholesterol was sprayed directly on the cornmeal. This cholesterol concentration was based upon previous studies which used 3% and 4% cholesterol-supplemented diets to increase the serum cholesterol concentrations in rats (Nikolova-Karakashian and Mariana 1991; Veiga and others 1995). Group III was given distilled water which contained 250 mg Pb²⁺/l as nitrate salt. Group IV was fed the cholesterol-supplemented diet and given the lead contaminated water. We chose this concentration of lead because previous studies have shown similar concentrations of lead in drinking water mimic blood Pb concentrations in United States residents with low to moderate Pb exposures (Bogden and others 1991; Bogden and others 1995). In addition, this concentration has been employed in other investigations to study the effects of dietary lead exposure (Bogden and others 1991; Bogden and others 1995; Han and others 1996; Zheng and others 1996). Lead nitrate was used in order to be consistent with previous studies (Tewari and others 1987; Devi and Fingerman 1995). The lead concentration of the present investigation approximates the daily lead exposure used by Jehan and Motlag (1995) to demonstrate that lead injections depress cholesterol concentrations in erythrocyte membranes. The volume of the food and water ingested by each animal was measured to verify that groups I and II were exposed to equal amounts of cholesterol and that groups III and IV were exposed to equal amounts of lead. Each animal was housed individually in a 26.7 cm x 48.3 cm x 20.3 cm polypropylene cage at a controlled temperature of 21 ± 1° C and a 12:12 hour light:dark cycle.

Animals were maintained on their respective regimen for a period of 35 days. At day 7 and every 7th day following, a 1.5 ml blood sample was taken by modification of the tail sectioning method of Liu and others (1996). Each animal was placed in a polyethylene box located on a surgical warming table for 3 min to encourage vasodilation of the caudal veins. The animal was then placed in a restrainer, the caudal vein was nicked with a razor blade, and a 1.5 ml blood sample was collected in an Eppendorf tube. Approximately 60 µl

of additional blood was collected in a capillary tube and used to measure packed cell volume. After the blood was collected, pressure was applied to the tail with sterile gauze until the bleeding stopped. This method and volume of blood collection is within acceptable limits for repeated blood collection for the rat (Flecknell 1987).

Upon collection of the blood, the capillary tube was centrifuged for 3 min. The blood was allowed to clot in the Eppendorf tube for 10 min and then centrifuged at 700 g for 10 min in order to isolate the serum. The serum was placed in another Eppendorf tube and refrigerated until it was analyzed.

Serum cholesterol concentrations were measured within 8 h of collection using a diagnostic kit available from Sigma Chemical Company, St. Louis, MO (Sigma No. 352-20). Serum hemoglobin and total protein concentrations were measured within 72 h of collection using a diagnostic kit for hemoglobin (Sigma No. 527A), and the Bradford assay (Bradford 1976) for total protein concentration. All assays employed a Shimadzu UV160U spectrophotometer and Biorad disposable cuvettes.

All data from the first week of this investigation were lost because of a technical problem with the centrifuge. Consequently, the week one data were omitted from this investigation. Packed cell volume, serum hemoglobin, protein, and cholesterol were analyzed using a two-way ANOVA with treatment groups and time as main effects. Specific differences among means ($p < 0.05$) were analyzed using the Tukey HSD post-hoc test. All statistical analyses were performed using a commercially available statistical software package (Statistica by Stat-Soft, Tulsa, OK).

RESULTS

The mean serum cholesterol concentration of each treatment group remained within the range of 66.7-90.9 mg/dl throughout the five week study period. Analysis of variance revealed that neither differences in the duration of dietary treatment (intragroup comparison between weeks) nor differences in dietary treatment (intergroup comparisons within each week) resulted in a significant difference in mean serum cholesterol concentration ($p > 0.05$) (Fig. 1).

Mean packed cell volume of the lead + cholesterol supplemented group was depressed 2.5% from that of the control group following two weeks of exposure to the respective dietary regimens (Fig. 2). The packed cell volume of the lead + cholesterol treatment group remained depressed from that of the control group throughout the first four weeks of the investigation, but this difference was only significant following three weeks of dietary exposure ($p < 0.05$). The mean packed cell volume of the lead + cholesterol group steadily increased until it approached the control level of 49.5% following the fifth week of dietary treatment.

Mean serum hemoglobin concentration fluctuated within the range of 7.8-21.1 mg/dl for all treatment groups. The mean hemoglobin concentration in the serum of the lead-exposed or cholesterol-exposed group was not significantly different from that of the control

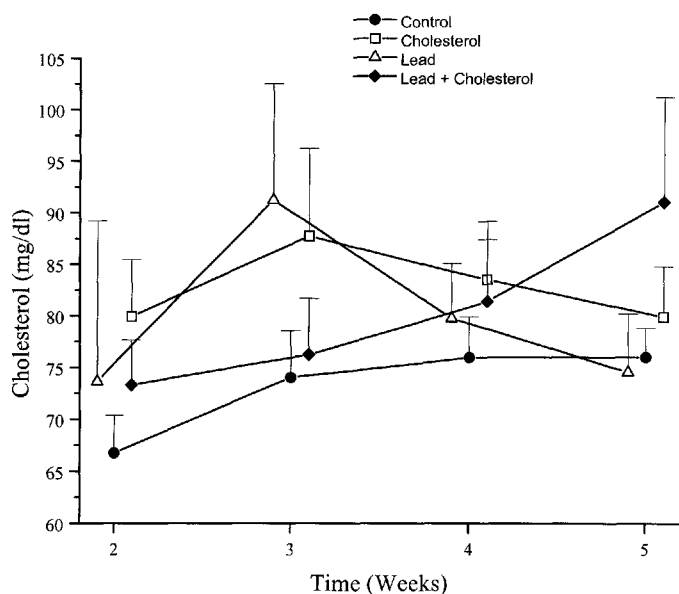


FIGURE 1. Changes in serum cholesterol concentrations of Sprague-Dawley rats over a five week period of exposure to 250 mg/l of dietary lead and/or a diet supplemented with 4% cholesterol. Bars represent S.E.M. and $n = 8$ for all data points. Values are offset from the week for visual acuity.

group at any point in the investigation ($p > 0.05$) (Fig. 3). Similarly, the dietary consumption of both lead and cholesterol had no significant impact ($p > 0.05$) upon the mean serum hemoglobin concentration.

DISCUSSION

The present investigation indicated that hemolysis did not occur as a result of lead-induced cholesterol deficiency. Mean serum cholesterol concentration of the

lead-exposed group was not depressed, indicating that lead exposure did not modify cholesterol metabolism (Fig. 1). Goodman and Noble (1968) found that the liver, plasma, and erythrocytes comprise a pool of cholesterol that is rapidly and continuously turning over (in terms of hours to days). Consequently, changes in serum cholesterol (or absence of changes) reflect changes in the cholesterol content of erythrocyte membranes and the liver. Cholesterol concentration within the erythrocyte membrane was not directly measured in the present investigation. In light of the work of Goodman and Noble (1968), it can be inferred from our results that the cholesterol content of erythrocyte membranes was not depressed. Consequently, the lead-exposed rats were not more susceptible to cholesterol deficiency-induced hemolysis than were the control rats.

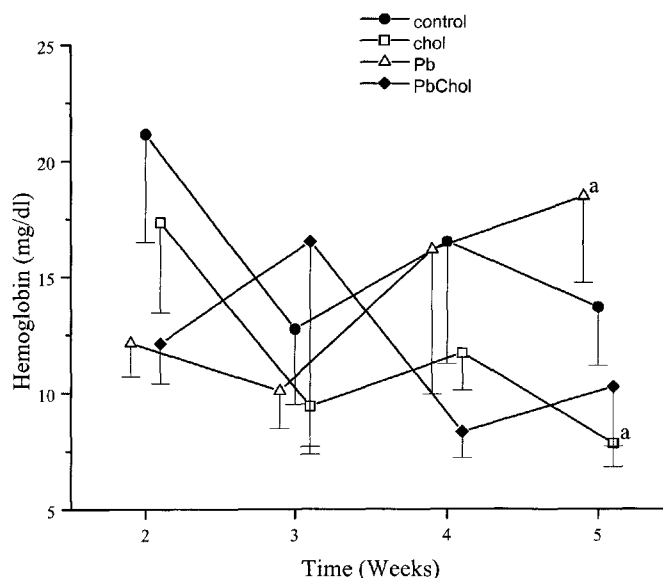


FIGURE 3. Changes in serum hemoglobin concentrations of Sprague-Dawley rats over a five week period of exposure to 250 mg/l of dietary lead and/or a diet supplemented with 4% cholesterol. Bars represent S.E.M. and $n = 8$ for most groups. $N = 7$ for Pb week 2, Pb and cholesterol week 3, and week 4 cholesterol and Pb and cholesterol. $N = 6$ for cholesterol week 2 and Pb week 3. An "a" designates a significant difference ($p < 0.05$) from the control value using the Tukey HSD post-hoc analysis. Values are offset from the week for visual acuity.

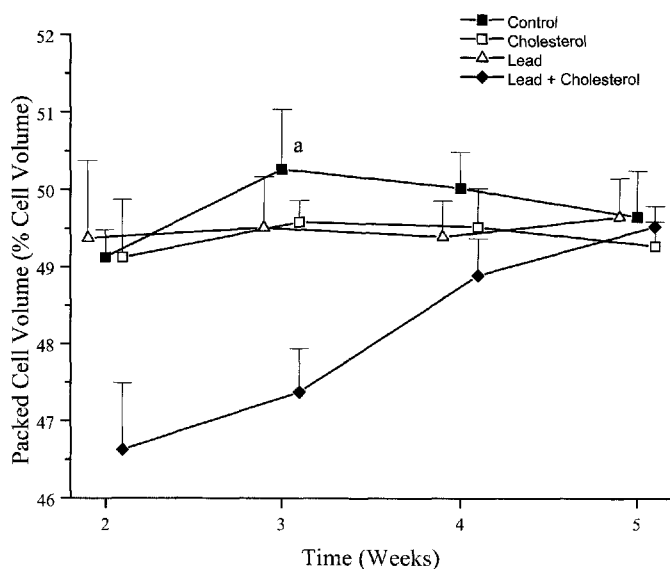


FIGURE 2. Changes in packed cell volume of venous blood from Sprague-Dawley rats over a five week period of exposure to 250 mg/l of dietary lead and/or a diet supplemented with 4% cholesterol. Bars represent S.E.M. and $n = 8$ for all groups except for the cholesterol week 3 where $n = 7$. An "a" designates a significant difference ($p < 0.05$) from the control value using the Tukey HSD post-hoc analysis. Values are offset from the week for visual acuity.

Measurement of packed cell volume (hematocrit) is a commonly used assay for monitoring hemolysis (Tewari and others 1987; Redondo and others 1995; Billy and others 1995). Mean packed cell volume of the lead-exposed group was not significantly depressed from that of the control at any time during the five week exposure period (Fig. 2). This indicated that hemolysis did not occur in these animals. It might be argued that hemolysis occurred at low levels and was masked by erythropoiesis to prevent the depression of packed cell volume. However, Grandjean and others (1989) found that lead exposure in man delays blood regeneration rather than promoting it. Furthermore, it was expected that increased hemolysis would result in increased serum hemoglobin concentrations. However, the mean concentration of hemoglobin in the serum of the lead-

exposed group was not significantly elevated above that of the control group at any point in our investigation (Fig. 3). Because serum hemoglobin concentrations above 20 mg/dl are indicative of hemolysis (Young and Bermes 1994), the absence of hemoglobin elevation in the present investigation is additional evidence that hemolysis did not occur. Haptoglobin is a 90 kilodalton protein which binds free hemoglobin and transports it to the liver for amino acid and iron recycling. The half-life of the hemoglobin-haptoglobin complex is only 90 minutes (Rand and others 1996). This suggests that if hemolysis occurred, hemoglobin was degraded too rapidly to accumulate in the serum.

The hypothesis of the present study was formulated as a result of the work of Dutta and Haghighi (1986) who found a significant depression in serum cholesterol concentration and packed cell volume of lead-exposed fish. However, the physiological changes that occurred in these lead-exposed fish were not observed in the rats of the present investigation. A comparison of these studies suggests that the route of lead exposure has an effect on the severity of the physiological responses or that there are species-specific differences in lead sensitivity. For example, the present investigation demonstrated that a 35-day dietary exposure to 250 mg Pb²⁺/l via the drinking water had no effect on the serum cholesterol concentration, serum hemoglobin concentration, or packed cell volume. In contrast, Ruparelia and others (1989) found that the serum cholesterol concentration of the fish *Oreochromis mossambicus* exposed to 18 mg Pb²⁺/l for 21 days were depressed by more than 50%. Tewari and others (1987) found that the serum cholesterol concentration of the fish *Barbus conchoniensis* exposed to 47.4 µg Pb²⁺/l for thirty days were depressed by 30.3%. Cholesterol concentrations in other organs of the same fish were depressed by 38.9% (liver) and 60.0% (testes). In addition, Tewari and others (1987) reported a 31.9% decrease in packed cell volume and a 14.9% decrease in serum hemoglobin concentration of lead-exposed fish. In contrast, the packed cell volume and serum hemoglobin concentration in the rats of the present investigation were not significantly depressed even though the lead concentration to which they were exposed was more than 5000 times greater than that to which the fish were exposed.

Toxicological differences between rats and fish may be explained by their differences in route of exposure. Perhaps fish were extremely sensitive to very low lead concentrations because they were continually exposed across their entire body surface area, including the vascular surface of the gills. In contrast, only the gastrointestinal tract of the rats was directly exposed to the lead source, and only a small proportion of ingested lead is absorbed across the gut (Foulkes and McMullen 1987). Because the proportion of lead that is absorbed across the lung epithelia is much greater than the proportion absorbed across the intestinal surface (relative to the amount of lead deposited on these structures) (Harrison and Laxen 1981), it is possible that an organism is more susceptible to the toxic effects of lead when this metal

is administered through the medium in which the organism lives rather than through the food or water it consumes.

In conclusion, the present investigation demonstrated that lead exposure did not induce a cholesterol deficiency sufficient to cause hemolysis. This was supported by the observations of normal serum cholesterol and hemoglobin concentrations as well as normal packed cell volume in lead-exposed rats. Finally, animals exposed to lead through the medium in which they live may be more vulnerable to the toxic effects of lead than those organisms exposed to lead through their diet.

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